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UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

LABORATORIOS MIRET, S.A.	
and	Civil No. 1:08-cv-04476-PKC
VEDEQSA, INC.	JUDGE CASTEL
Plaintiffs,	
v.	AMENDED COMPLAINT
A&B INGREDIENTS, INC.	JURY TRIAL DEMAND
Defendant.	

For its Amended Complaint against A&B Ingredients, Inc., plaintiffs Laboratorios Miret, S.A. and Vedeqsa, Inc. (collectively, "Plaintiffs") state as follows:

THE PARTIES

1. Plaintiff Laboratorios Miret, S.A. ("Lamirsa") is a corporation organized and existing under the laws of Barcelona, Spain. Lamirsa has a place of business at Geminis, 4, Polig. Ind. Can Parellada, 08228 Terrasa, Spain.

2. Plaintiff Vedeqsa, Inc. ("Vedeqsa") is a corporation organized and existing under the laws of the State of Delaware. Vedeqsa has a place of business at 11 Penn Plaza, 5th Floor, New York, New York, 10001.

3. Defendant A&B Ingredients, Inc. ("A&B") is a corporation organized and existing under the laws of the State of New Jersey, having a place of business at 24 Spielman Road, Fairfield, New Jersey, 07004. Upon information and belief, A&B regularly does business in this judicial district including through its acts of selling and offering products for sale in this district.

JURISDICTION AND VENUE

4. This action arises under the Patent Laws of the United States, Title 35, United States Code. This Court has jurisdiction under 28 U.S.C. § 1338(a), (b), and 28 U.S.C. § 1367.

5. Venue is proper in this district under 28 U.S.C. §§ 1391(b) and (c) and 1400(b).

FACTUAL BACKGROUND

6. Lamirsa is a developer of specialty chemical products in a wide variety of industries, including chemicals for use in the antimicrobial market.

7. Antimicrobial products or agents are used as a preservative to reduce the possibility of food borne illnesses by controlling the risk of microbial contamination in food, including meat products and poultry products, Ready-To-Eat dishes, pasta, juices, soft drinks, and dairy products, among others products.

8. Lamirsa manufactures Lauric Arginate (N^{α}-Lauroyl-L-arginine ethyl ester monohydrochloride), more commonly referred to as LAE. LAE is a cationic surfactant that has significant antimicrobial properties and is often referred to as a Lauric Arginate preservative.

Lamirsa also manufactures Mirenat-N®, a blend of LAE and other components for use as a food preservative and related preservatives.

9. Lamirsa invented a novel method of producing cationic surfactants, including LAE. Lamirsa's innovative method is protected by U.S. Patent No. 7,087,769 ("the '769 Patent"), attached as Exhibit A.

10. Lamirsa also invented a novel method for using cationic surfactants, including LAE, as food preservatives. Lamirsa's innovative method and resulting food product are protected by U.S. Patent No. 7,407,679 ("the '679 Patent"), attached as Exhibit B.

11. Lamirsa invested a substantial sum of money, time and resources in demonstrating the safety and effectiveness of LAE as a food preservative and pursued and obtained a notice from the U.S. Food and Drug Administration that LAE qualifies as a Substance Generally Recognized as Safe (GRAS notice), thus allowing its use in certain food products in the United States.

12. Lamirsa also developed significant proprietary know-how and confidential technical and business information relating to the effectiveness, use, potential applications and marketing of LAE based products ("LAE Trade Secrets"), including Miranet-N®, and spent substantial time, money and resources in developing a market for Lauric Arginate preservatives in the United States and elsewhere.

13. Vedeqsa is the exclusive licensee in the United States of the '769 Patent and the '679 Patent (collectively, "the Lamirsa Patents").

14. Vedeqsa is engaged in the promotion, sales, and marketing in the United States of antimicrobial products manufactured by Lamirsa, specifically Miranet-N®.

15. Upon information and belief, A&B promotes, manufactures, and sells a Lauric Arginate preservative under the trademark, CytoGuard, that includes LAE.

16. Upon information and belief, A&B manufactures and/or instructs others to manufacture LAE using a process that directly infringes claims of the '769 Patent.

17. Upon information and belief, A&B imports into the United States LAE manufactured elsewhere using a process that directly infringes claims of the '769 patent.

18. A&B has notice of and is aware of the '769 Patent.

19. Upon information and belief, A&B uses LAE and/or instructs others to use LAE in a manner that directly infringes claims of the '679 Patent.

20. Upon information and belief, A&B instructs others to use LAE to make food products that directly infringe claims of the '679 Patent.

21. A&B has notice of and is aware of the '679 Patent.

22. In 2004, A&B approached Lamirsa about becoming a distributor in the U.S. of LAE based food preservatives. Lamirsa and A&B executed a letter of intent in 2004 including a confidentiality provision preventing A&B from using or disclosing Lamirsa's proprietary information A&B learned from Lamirsa, including the LAE Trade Secrets.

23. Upon information and belief, prior to approaching Lamirsa, A&B did not have substantial experience in the use or sales of antimicrobial agents and food preservatives.

24. Upon information and belief, prior to approaching Lamirsa, A&B did not offer for sale an antimicrobial or food preservative product.

25. Lamirsa and A&B engaged in substantial negotiations through 2004 to 2006.

26. During this time period, Lamirsa invested a substantial amount of money, resources and time to sufficiently educate A&B and disclosed LAE Trade Secrets to A&B for the

sole purpose of facilitating A&B's effective and safe promotion and sales of LAE based products in the U.S. once A&B became a distributor of Lamirsa in the U.S.

27. Also during this time period, Lamirsa provided its Miranet-N® product to A&B for analysis and testing by potential customers and for A&B to become more familiar with the properties and use of LAE based products as food preservatives. Lamirsa's scientists consulted with A&B and provided A&B with the LAE Trade Secrets concerning LAE analysis and the preferred use of LAE.

28. Also during this time period, A&B improperly associated itself with Lamirsa's LAE product and promoted itself as the distributor of Lamirsa's LAE product in the U.S. manufactured using Lamirsa's patented process and promoted itself as knowledgeable in the use of Lamirsa's LAE Trade Secrets.

29. Upon information and belief, A&B continues to improperly associate itself with Lamirsa, with Lamirsa's LAE product and with Lamirsa's LAE Trade Secrets.

30. In mid 2006, negotiations between Lamirsa and A&B ended without reaching an agreement.

31. In 2007, A&B began promoting and selling its own version of Lamirsa's LAE product to the same customers and potential customers to which Vedeqsa promotes and sells Lamirsa's LAE product. Upon information and belief A&B instructs its customers and potential customers about the use of its LAE product using information learned from Lamirsa.

32. Upon information and belief, A&B was able to enter the U.S. market with its version of an LAE based product because of the LAE Trade Secrets it learned from Lamirsa substantially sooner than it otherwise would have been able to do so.

33. Upon information and belief, it is inevitable that A&B will use and continue to use Lamirsa's LAE Trade Secrets because A&B did not have substantial knowledge about the effectiveness, use, or promotion of an LAE product before learning the LAE Trade Secrets from Lamirsa.

34. Upon information and belief, A&B continues to promote itself as a source of products manufactured using the process of Lamirsa's '769 Patent.

COUNT I: INFRINGEMENT OF THE '769 PATENT

35. Plaintiffs incorporate by reference herein the allegations of Paragraphs 1-34 of this Complaint.

36. Lamirsa is the owner by assignment of the '769 Patent entitled PROCESS FOR THE PREPARATION OF CATIONIC SURFACTANTS. The '769 Patent was duly and legally issued by the United States Patent and Trademark Office ("USPTO") on August 8, 2006. The '769 Patent is still in force and effect and is presumed valid under the U.S. patent laws.

37. Vedeqsa is the exclusive licensee in the U.S. of the '769 Patent.

38. A&B has been and still is directly infringing the '769 Patent under 35 U.S.C. § 271(g) by importing a product made by a process claimed in the '769 Patent.

39. As a result of A&B's infringement, Plaintiffs have suffered monetary damages in an amount not yet determined, and will continue to suffer irreparable harm in the future unless A&B's infringing activities are enjoined by this Court.

40. Plaintiffs will be greatly and irreparably harmed unless preliminary and permanent injunctions are issued enjoining A&B and its agents, servants, employees, attorneys, representatives, and all others acting on its behalf from infringing the '769 Patent.

COUNT II: INFRINGEMENT OF THE '679 PATENT

41. Plaintiffs incorporate by reference herein the allegations of Paragraphs 1-40 of this Complaint.

42. Lamirsa is the owner by assignment of the '679 Patent entitled USE OF CATIONIC PRESERVATIVE IN FOOD PRODUCTS. The '679 Patent was duly and legally issued by the USPTO on August 5, 2008. The '679 Patent is still in force and effect and is presumed valid under the U.S. patent laws.

43. Vedeqsa is the exclusive licensee in the U.S. of the '679 Patent.

44. A&B has been and still is directly infringing the '679 Patent under 35 U.S.C. § 271(a) by importing a product made by a process claimed in the '679 Patent.

45. A&B has been and still is indirectly infringing the '679 Patent under 35 U.S.C. § 35 U.S.C. § 271(b) by instructing its customers to make products that directly infringe claims of the '679 Patent and by instructing its customers to make products using processes that directly infringe claims of the '679 Patent.

46. A&B has been and still is indirectly infringing the '679 Patent under 35 U.S.C. § 35 U.S.C. § 271(c) by selling components of products that directly infringe claims of the '679 Patent.

47. As a result of A&B's infringement, Plaintiffs have suffered monetary damages in an amount not yet determined, and will continue to suffer irreparable harm in the future unless A&B's infringing activities are enjoined by this Court.

48. Plaintiffs will be greatly and irreparably harmed unless preliminary and permanent injunctions are issued enjoining A&B and its agents, servants, employees, attorneys, representatives, and all others acting on its behalf from infringing the '679 Patent.

COUNT III: UNFAIR COMPETITON – NEW YORK COMMON LAW

49. Plaintiffs incorporate by reference herein the allegations of Paragraphs 1-48 of this Complaint.

50. The acts of A&B as described above constitute unfair competition in violation of Plaintiffs' rights under the common law of the State of New York.

51. As a result of the acts of A&B's as alleged herein, Plaintiffs have suffered and will continue to suffer great damage to Plaintiffs' business, goodwill, reputation, and profits.

COUNT IV: THEFT OF TRADE SECRETS

52. Plaintiffs incorporate by reference herein the allegations of Paragraphs 1-51 of this Complaint.

53. Lamirsa owns and maintains the LAE Trade Secrets, and the LAE Trade Secrets have independent economic value to Plaintiffs.

54. A&B has acquired the LAE Trade Secrets under an obligation of confidentiality and is using the LAE Trade Secrets improperly contrary to its obligations resulting in a misappropriation of the LAE Trade Secrets.

55. A&B's misappropriation of the LAE Trade Secrets has been willful, wanton and/or reckless.

56. As a result of A&B's misappropriation of the LAE Trade Secrets, Plaintiffs have suffered monetary damages in an amount not yet determined, and will continue to suffer irreparable harm in the future unless A&B's misappropriation is enjoined by this Court.

57. Plaintiffs will be greatly and irreparably harmed unless preliminary and permanent injunctions are issued enjoining A&B and its agents, servants, employees, attorneys, representatives, and all others acting on its behalf from using the LAE Trade Secrets.

PRAYER FOR RELIEF

Plaintiffs pray for the following relief:

(a) A judgment that A&B has directly infringed and continues to infringe the Lamirsa Patents;

(b) A judgment against A&B awarding Plaintiffs damages suffered by Plaintiffs pursuant to 35 U.S.C. § 284 on account of A&B's infringement of the Lamirsa Patents;

(c) A preliminary injunction pursuant to 35 U.S.C. § 283 enjoining A&B and any entity acting in concert with A&B from infringing the Lamirsa Patents;

(d) A permanent injunction pursuant to 35 U.S.C. § 283 enjoining A&B and any entity acting in concert with A&B from infringing the Lamirsa Patents;

(e) A judgment that this is an exceptional case and that Plaintiffs be awarded treble damages, reasonable attorney fees, and expenses pursuant to 35 U.S.C. § 285;

(f) A judgment against A&B awarding Plaintiffs damages suffered by Plaintiffs as a result of A&B's unfair competition;

(g) A judgment against A&B awarding Plaintiffs damages suffered by Plaintiffs as a result of A&B's theft of the LAE Trade Secrets;

(h) Preliminary and permanent injunctions enjoining A&B and its agents, servants, employees, attorneys, representatives, and all others acting on its behalf from using the LAE Trade Secrets;

(i) A judgment that A&B be directed to pay Plaintiffs their costs incurred herein and such other and further relief as the Court deems just and equitable.

Date: August 15, 2008

Respectfully submitted,

s/ Mark C. Johnson
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JURY DEMAND

Plaintiffs Laboratorios Miret, S.A. and Vedeqsa, Inc. respectfully request a trial by jury as to all issues so triable.

Respectfully submitted,

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CERTIFICATE OF SERVICE

The undersigned, a member of the Bar of this Court, hereby certifies that on the 15th day of August, 2008, a copy of the within ***Amended Complaint*** was served upon the following by *federal express*:

President
Purac America Inc.
111 Barclay Boulevard
Lincolnshire, Illinois 60069

Lauren M. Dayton

Lauren M. Dayton

US007087769B1

(12) United States Patent
Contijoch Mestres et al.

(10) Patent No.: US 7,087,769 B1
(45) Date of Patent: Aug. 8, 2006

(54) **PROCESS FOR THE PREPARATION OF CATIONIC SURFACTANTS**

(75) Inventors: **Agustin Contijoch Mestres**, deceased, late of Barcelona (ES); by **Alex Contijoch Manent**, legal representative, Barcelona (ES); by **Monica Contijoch Manent**, legal representative, Barcelona (ES); by **Maria Contijoch Manent**, legal representative, Barcelona (ES); **Javier Rodriguez Martinez**, Terrassa Barcelona (ES); **Joan Seguer Bonaventura**, Barcelona (ES)

(73) Assignee: **Laboratorios Miret, S.A.**, Barcelona (ES)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 64 days.

(21) Appl. No.: **10/467,780**

(22) PCT Filed: **Jun. 3, 2000**

(86) PCT No.: **PCT/EP00/05072**

§ 371 (c)(1),
(2), (4) Date: **Nov. 26, 2004**

(87) PCT Pub. No.: **WO01/94292**

PCT Pub. Date: **Dec. 13, 2001**

(51) **Int. Cl.**
C07C 231/00 (2006.01)

(52) **U.S. Cl.** **554/69**; 554/68

(58) **Field of Classification Search** 554/168, 554/169
See application file for complete search history.

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

EP 749960 * 12/1996

OTHER PUBLICATIONS

PCT International Search Report for subject application, issued Nov. 28, 2001 w/ following attachments: *J. Chem. Soc. Perkin Trans.* 1 1990 XP-000972986; and *Database Beilstein*, XP-002156958, vol. 41, No. 10, 1986.

* cited by examiner

Primary Examiner—Deborah D. Carr
(74) *Attorney, Agent, or Firm*—John W. Renner; Renner, Otto, Boisselle & Sklar

(57) **ABSTRACT**

The invention concerns the preparation of cationic surfactants derived from the condensation of an acid, preferably a fatty acid or a hydroxy acid with a number of carbon atoms of 8–14 with esterified amino acids, preferably basic-type amino acids, more preferably (L)-arginine. The method comprises a first step in which the esterifiction of the amino acid with an alcohol is performed and a second step for the condensation with a chloride of an acid, preferably an acyl chloride of a fatty acid or a hydroxy acid, whereby the second step is performed in an aqueous environment with a pH value between (6 and 7), preferably between (6, 7) and (6, 9).

9 Claims, No Drawings

EXHIBIT
A

PROCESS FOR THE PREPARATION OF CATIONIC SURFACTANTS

INTRODUCTION

The present invention relates to a new process for the preparation of cationic surfactant products, the hydrophilic portion of which consists of an esterified amino acid, preferably an esterified basic-type amino acid and the hydrophobic portion thereof consists of an acid, preferably a fatty acid or a hydroxy acid linked to the amino group of the amino acid via an amide bond.

BACKGROUND OF THE INVENTION

Cationic surfactant compounds are well-known in the art for their capacity to inhibit the formation of bacterial colonies.

This antimicrobial activity is described in detail in EP-A-0 749 960. The efficacy of the product lauramide of L-arginine ethyl ester monohydrochloride was proven against more microorganisms: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. The product is further known to be efficaceous against the bacteria *Alcaligenes faecalis, Bordetella bronchiseptica, Citrobacter freundii, Enterobacter aerogenes, Klebsiella pneumoniae spp. pneumoniae, Proteus mirabilis, Salmonelly thyphimurium, Serratia marcescens, Bacillus subtilus, Bacillus cereus spp. mycoide, Micrococcus luteus, Arthrobacter oxydans, Mycobacterium phlei* and *Listeria monocytogenes*, against the yeasts *Rhodotorula rubra, Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* and and against the fungi *Mucor rouxii, Aureobasidium pullulans, Chaetonium globosum, Gliocadium virens, Penicillium chrysogenum* and *Penicillium funiculosum*. It is the particular advantage of the product, that it displays an excellent efficacy against these microorganism strains and is well tolerated by animals and human beings. This positive safety aspect makes the product highly suitable for any use leading to direct contact with the human body, like in cosmetic preparations and in the food industry.

The preparation of the cationic surfactant compounds with antimicrobial activity is described in the prior art.

The method described in ES-A-512643 is related to a first step of preparing an ester from the basic type amino acid and an alcohol and in the second step performing a condensation of the ester with a fatty acid to obtain the final product. It is a typical aspect of the method, that initially a solution of the catalyst thionyl chloride in the alcohol is prepared and that the amino acid is added to this solution. Heating of the solution is required and it takes at least 16 hours to bring the reaction to an end. The second step of the condensation is performed by adding the fatty acid as the free acid to the solution in the presence of a coupling reagent such as dicyclohexylcarbodiimide (DCDD).

An improved method has been provided in EP-A-0 749 960 which is differing from the previously mentioned method by providing in the first step a dispersion of the basic-type amino acid in alcohol and adding a catalyst like thionyl chloride to this dispersion in a drop-wise manner. It is the advantage of this adaptation of the method, that this drop-wise addition allows an excellent control of the reaction without the need of applying external heat to make the reaction run. A further difference is the performance of the second step by using a fatty acid halide. It is a particular advantage that this adaptation allows the performance of the reaction in an aqueous environment, which is a particular advantage, when the use of the final product is intended to be in the food industry. When the thionyl chloride is added, then arginine is solubilised for the formation of arginine ethyl ester dihydrochloride.

The method described in EP-A-0 749 960 is further characterised by the fact, that the second step of the condensation of the esterified amino acid is performed in an alkaline environment. EP-A-0 749 960 describes the need to perform the condensation at an alkaline pH, preferably at a pH between 8 and 10. The reason for using the alkaline environment is evidently the conviction in the art, that this type of reaction, which is a Schotten-Baumann reaction requires an alkaline environment. A comparable reaction is described in GB-A-1 352 420 which describes the reaction of arginine with a higher aliphatic acyl halide and likewise indicates the presence of an alkaline aqueous medium. A specific example contained in this prior art document indicates a pH value of 11.5–12.0 adjusted with sodium hydroxide.

The process described in EP-A-0 749 960 allows a relatively fast and efficient preparation of the wanted cationic surfactants to be used as antimicrobial products, but the inventors of the present invention have set themselves the task to continuously improve the preparative method in order to be able to produce the products industrially in the required quality in an economic manner. This continuous evaluation of improvements of the method has finally led to the present invention.

DESCRIPTION OF THE INVENTION

The present invention is directed to a novel method for the preparation of cationic surfactants suitable for antimicrobial use in cosmetics and food preparations. The inventive method can be used for the preparation of compounds prepared from any type of amino acid, preferred cationic surfactants prepared according to the inventive method are derived from basic-type amino acids, like (L)-lysine and (L)-arginine, particularly preferred is the amino acid (L)-arginine.

The amino acid, preferably the basic-type amino acid and even more preferably (L)-arginine is reacted in a first step of the inventive method with an alcohol to form the corresponding ester compound. The type of alcohol is not essential for the inventive method, but the preferred type of alcohol is an alcohol containing 1 to 12 carbon atoms whereby the alcohol can be linear or branched. Examples of such alcohols are methanol, ethanol, propanol, isopropanol, 1-butanol, 2-butanol, tert-butanol, pentanol, hexanol; heptanol, octanol, nonanol, decanol, undecanol and dodecanol. The preferred type of alcohol is ethanol, which is not only particularly suitable for the inventive method but is also preferable for the preparation of cationic surfactants to be used in the food industry while being well tolerated and being essentially free of toxic side effects.

The preferred way of preparing in the first step an ester from the amino acid and the alcohol in the inventive method corresponds to the method disclosed in EP-A-0 749 960. In this first step of the preparation a solution or dispersion of the amino acid in the alcohol is prepared and according to the preferred method a dispersion of the basic type amino acid in ethanol. The amino acids including the basic type amino acids are usually soluble in alcohols. However, the amino acid L-arginine monohydrochloride is not soluble in ethanol and for that reason a dispersion of this particular amino acid in ethanol is prepared to be the initial preparation of the inventive reaction.

To this solution or dispersion of the amino acid in the alcohol a suitable catalyst is added in a highly controlled manner. Any type of conventional catalyst can be used in this esterification step, like catalysts sulphuryl chloride, hydrogen chloride, phosphorus trichloride and phosphorus pentachloride, but the compound thionyl chloride has turned out to be particularly suitable as a catalyst. The catalyst, for instance thionyl chloride is added over a total period of two hours.

The total amount of the catalyst depends on the specific conditions of the reaction. In the method described in EP-A-0 749 960 it has been stated, that a total amount of 1.3 equivalents thionyl chloride is added to 1 equivalent of dispersed (L)-arginine, it has now been found out, that the highly specific amount of 1.27 equivalent of thionyl chloride leads to an optimum preparation of the ester, when the ester is formed from arginine with ethanol. The reason why this specific relative amount leads to the optimum final result is not clear at the present time. It has turned out, that in the industrial environment the catalyst thionyl chloride is added at a rate of 140 kg/h to 164 kg/h to obtain a final amount of the L-arginine ethyl ester dihydrochloride of 2100 kg of the crude final product, the purity of this crude product usually being between 90 and 95 %.

The controlled addition of thionyl chloride leads to a regular heat generation in the exothermic reaction which makes it possible to perform the reaction without heating from an external heat source. In particular in industrial production this way of performing the method is of great economic advantage.

The duration of the esterification reaction depends on a number of circumstances, in particular on the compounds used as constituents for the preparation of the ester. The conditions of the method for this part of the preparation allow a very fast preparation of the ester, a duration of 3 to 6 hours to finish the reaction is usual.

After the completion of the esterification reaction a final product is obtained which is usually a hydrochloride, in the case of the basic type amino acids usually a dihydrochloride. The product is crude, containing a number of further constituents such as a certain amount of the unreacted amino acid. The presence of such impurities is of no particular concern, purification can be performed but is certainly not necessary. Furthermore the yield of this esterification reaction is very good, in case of preparing the ester from the amino acid arginine and ethanol the yield is usually much higher than 90%, specific yields of 96% being regularly observed.

The product of the first step of the preparation, in more or less purified form, is obtained as an oily product. The solvent used in the esterification reaction of the invention is the alcohol, which solvent is removed carefully in order to avoid any unwanted effects during the second step of the reaction. Any kind of conventional method for the removal of the solvent is suitable, none is particularly preferred. The most regularly used method is the evaporation of the solvent under reduced pressure, under laboratory as well as industrial conditions. The purity of the product obtained in the first step is usually between 90 and 95% of the compound arginine ethyl ester dihydrochloride.

In the second step of the inventive method the esterified compound is further reacted with a carboxylic acid chloride to obtain the corresponding amide of the esterified amino acid. Basically any kind of acid chloride can be used in the inventive method, but fatty acid chlorides or hydroxy acid chlorides with a total number of carbon atoms between 8 and 14 are preferred and even more preferred linear chain fatty

acid chlorides and hydroxy acid chlorides with a total number of 8 to 14 carbon atoms. Examples of such fatty acids are lauric acid, caprylic acid, caprylic acid, myristic acid and palmitic acid. Particularly preferred is lauroyl chloride, not only for the excellent performance in the reaction, but also for its excellent toxicological history.

It is one of the characteristics of the inventive method that this second step of the reaction is performed in an aqueous environment without the presence of any organic solvent. There are numerous possible uses of the final product, for which the presence of a minor amount of an organic solvent is of no particular concern, but as has been mentioned above repeatedly one of the specific intended uses of the products prepared according to the inventive method are in the food industry and any presence of organic components is unwanted under all circumstances. The preparation of the aqueous solution can be performed by stirring the ester of the amino acid in a suitable amount of water. As such water normal demineralised water, deionised water and destined water may be used, preferred is the use of deionised water.

It is one of the specific effects of the inventive method, that the pH value during the second step of the reaction is not kept in the alkaline pH range as was the case in the conventional way of preparing the product, but rather in a practically neutral pH range of 6.7–6.9. Numerous investigations have been performed by the inventors of the present invention during which it turned out, that in particular at this pH range the optimum values of the reaction yield are observed. A reaction yield of more than 90% can easily be obtained under these conditions which is a significant improvement over the yield obtained under the conventional reaction conditions. It is a further logical aspect of the inventive method, that the amount of impurities detected under these conditions is lower than under the conventional reaction conditions.

The dissolution of the reaction product obtained in the first step of the reaction leads to an aqueous solution of acidic character. According to the inventive process it is required to bring this pH value to a final value of 6.7–6.9 as the optimum pH range to perform the condensation reaction. This adjustment of the pH value can be performed with any basic product, as solution or alternatively by adding a dry basic compound. Addition of a solution is the most simple method and easiest to handle to obtain a precise and exact pH value under industrial conditions.

The type of basic product used to bring the pH value into the preferred range is of no particular importance, any kind of basic product may be used. In usual practice, the use of alkali metal hydroxides like sodium or potassium hydroxide is preferred, in particular of sodium hydroxide.

After adjusting the pH value to the wanted level, in particular to the pH level of between 6.7 and 6.9, the temperature of the reaction mixture is brought to a suitable level for the performance of the reaction. In the prior art the temperature was evidently not considered to be one of the key parameters since regularly the only indication found is the temperature to be below a level of 20° C., a more precise definition of the temperature apparently to be considered as of no particular concern. It is one further unexpected result obtained by the inventors of the present invention, that the temperature played a significant role in the determination of the final result of the reaction. A temperature between 10 and 15° C. turned out to be particularly suitable for the performance of the reaction, in particular since the obtained final amide turned out to display the highest purity of the obtained final amide. This optimum temperature of 10–15° C. is kept during the complete second step of the reaction.

The amidation reaction is started by the addition of the chloride of the fatty acid or of the hydroxy acid. The total amount of the chloride of the fatty acid or the hydroxy acid is 0.96 equivalent (per 1 equivalent of the esterified amino acid) instead of 1.1 equivalent as was indicated in the prior art.

The duration of the amidation reaction is 5 to 10 hours, a duration of 6 h is usual. When the condensation is performed, the final product is recovered by means of centrifugation of the precipitated product. On the conventional preparation method the pH had to be adjusted at the end of the preparation to a pH between 6 and 7, this additional adjusting step is now not required any more.

The final preparation of the product is performed with usual methods.

EXAMPLE

The method for the preparation of the cationic surfactant according to the invention displays a number of similarities with the method described in EP-A-0 749 960.

First Step

Preparation of L-arginine ethyl ester dihydrochloride.

In a glass reactor with a capacity of 2 liters with a five-socket lid and provided to with a mechanical stirrer, reflux condenser, nitrogen gas inlet, dropping funnel and thermometer, 1 equivalent of L-arginine hydrochloride is suspended in 200 ml of essentially water-free ethyl alcohol at room temperature and the stirring is started.

The catalyst thionyl chloride is added drop-wise in a total amount of 1.27 equivalents over a period of two hours, reflux conditions being maintained by additional heating. After the reaction mixture has reached the boiling point, stirring is continued for three further hours, after which the reaction is completed.

The solvent is removed by evaporation at reduced pressuer repeatedly, with intermediate additions of dry ethanol.

Second Step

Preparation of the lauramide of L-arginine ethyl ester monohydrochloride.

The crude reaction product obtained in the first step is dissolved in water and the pH of the solution is brought to a specific pH value by the addition of aqueous sodium hydroxide. The reaction conditions are investigated under conditions where the final pH of the reaction solution is between 4.5 and 12 (inclusive). The pH of the reaction is carefully kept constant at this value until completion of the reaction.

To the solution 0.96 equivalent of lauroyl chloride is added drop-wise, whereby the temperature of the mixture is kept at a temperature of 10–15° C. by means of an appropriate cooling bath containing ethylene glycol.

After completion of the reaction, the stirring is maintained for a further two hours, after which the pH of the solution is adjusted to a final value of 6–7 with hydrochloric acid or sodium hydroxide. Finally, the crude reaction product is filtered off, whereby a white solid composition of pearly appearance is obtained.

The obtained reaction product is analysed with standard chromatographic procedures in order to obtain the amount of the final product and the amounts and type of impurities present in the final product. The reaction yield was calculated.

The obtained data are displayed in the following table 1.

TABLE 1

	IMPURITIES			
pH value	LAE (%, w/w)	LAS (%, w/w)	LAURIC ACID (%, w/w)	REACTION YIELD
4.5	54	0.5	5.6	55–58
5.0	62	0.6	5.2	63–66
5.5	75	0.8	4.6	76–79
6.0	79	0.9	3.7	81–83
6.5	83	1	3.0	84–87
6.7–6.9	89	1	3.5	90–95
7.0	86	1	3.5	88–90
7.5	82	3	3.7	83–87
8.0	78	4	4.2	79–82
8.5	74	6	4.8	75–78
9.0	70	9	5.1	71–74
9.5	67	11	5.2	69–71
10.0	63	22	5.4	64–67
11	58	33	5.7	59–62
12	39	47	5.9	40–42

Explanation of abbreviations.
LAE ethyl ester of N^{α}-lauroyl-L-arginine monohydrochloride
LAS N^{α}-lauroyl-L-arginine
W/w weight/weight

The invention claimed is:

1. Method for the preparation of cationic surfactants derived form the condensation of an acid with an esterified amino acid comprising the esterification of the amino acid with an alcohol in a first step and in a second step performing the condensation with a chloride of an acid in an aqueous solution, characterised in that;

the second step is performed at a pH value between 6 and 6.9.

2. Method according to claim **1**, whereby the temperature in the second step of the method is kept at 10–15° C.

3. The method according to claim **1**, wherein said amino acid is a basic-type amino acid.

4. A method according to claim **2**, wherein said amino acid is a basic-type amino acid.

5. The method of claim **4**, wherein said amino acid is (L)-arginine.

6. The method of claim **1**, wherein said acid is a fatty acid or an hydroxy acid with a number of carbon atoms of 8–14.

7. A method according to claim **5**, wherein said acid is a fatty acid or an hydroxy acid with a number of carbon atoms of 8–14.

8. A method according to claim **3**, wherein said acid is a fatty acid or an hydroxy acid with a number of carbon atoms of 8–14.

9. The method of claim **1**, whereby the pH value is between 6.7 and 6.9.

* * * * *

US007407679B2

(12) United States Patent
Beltran et al.

(10) Patent No.: US 7,407,679 B2
(45) Date of Patent: Aug. 5, 2008

(54) **USE OF CATIONIC PRESERVATIVE IN FOOD PRODUCTS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/493,783**

(22) PCT Filed: **Oct. 25, 2001**

(86) PCT No.: **PCT/EP01/12358**

§ 371 (c)(1),
(2), (4) Date: **Apr. 26, 2004**

(87) PCT Pub. No.: **WO03/034842**

PCT Pub. Date: **May 1, 2003**

(65) **Prior Publication Data**

US 2004/0265443 A1 Dec. 30, 2004

(51) **Int. Cl.**
A21D 4/00 (2006.01)
A23L 3/3463 (2006.01)

(52) **U.S. Cl.** **426/335**; 426/321

(58) **Field of Classification Search** 426/335, 426/321

See application file for complete search history.

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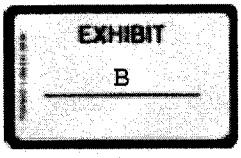
(57) **ABSTRACT**

A novel use of cationic preservatives and preparations according to this novel use. A cationic preservative derived from lauric acid and arginine, in particular, the ethyl ester of the lauramide of the arginine monohydrochloride, hereafter named LAE, can be used for the protection against the growth of microorganisms. LAE and related compounds are particularly suitable to be used in the preservation of all perishable food products. The composition optionally comprises auxiliary components and excipients.

12 Claims, No Drawings



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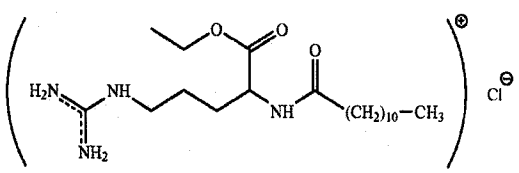
USE OF CATIONIC PRESERVATIVE IN FOOD PRODUCTS

This application is a national phase of International Application No. PCT/EP01/12358 filed Oct. 25, 2001 and published in the English language.

This invention relates to a novel use of cationic preservatives and preparations according to this novel use.

Despite the food industry must avoid the use of preservative products by means of good manufacture practices as it is described in the national and international regulations, it is often necessary to warrant the needed storage capability of the produced food-stuff but never to hide defective effects of a manipulation or manufacture technique.

A cationic preservative derived from lauric acid and arginine, in particular, the ethyl ester of the lauramide of the arginine monohydrochloride, hereafter named LAE, could be used for the protection against the growth of the microorganisms. The chemical structure is described in the following formula:



The preparation of this product has been described in Spanish patent application ES-A-512643.

Biological studies carried out at different research centres under supervision of the present applicant showed LAE to act mainly over the external and cytoplasmatic membrane of the microorganisms and, also, into the cytoplasmatic media, preventing their proliferation. Its action depends on the kind of microorganism and on the exposure time.

Besides, its metabolism in rats has been studied showing a fast absortion and metabolisation into naturally-occurring amino acids and the fatty acid lauric acid, which are eventually excreted as carbon dioxide and urea. Toxicological studies have demonstrated, that LAE is completely harmless to animals and humans.

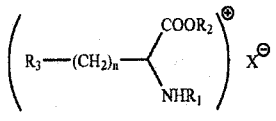
Therefore, LAE and related compounds are particularly suitable to be used in the preservation of all perishable food products.

This compound is remarkable for its inhibitory action over the proliferation of different microorganisms, such as bacteria, fungi and yeasts. The minimum inhibitory concentrations of LAE are shown in the following table 1.

TABLE 1

Kind	Microorganism	M.I.C. (ppm)
Gram + Bacteria	*Arthrobacter oxydans* ATCC 8010	64
	Bacillus cereus var mycoide ATCC 11778	32
	Bacillus subtilis ATCC 6633	16
	Clostridium perfringens ATCC 77454	16
	Listeria monocytogenes ATCC 7644	10
	Staphylococcus aureus ATCC 6538	32
	Micrococcus luteus ATCC 9631	128
	Lactobacillus delbrueckii ssp lactis CECT 372	16
	Leuconostoc mesenteroides CETC 912	32
Gram – Bacteria	*Alcaligenes faecalis* ATCC 8750	64
	Bordetella bronchiseptica ATCC 4617	128
	Citrobacter freundii ATCC 22636	64
	Enterobacter aerogenes CECT 689	32
	Escherichia coli ATCC 8739	32
	Escherichia coli 0157H7	20
	Klebsiella pneumoniae var pneumoniae CECT 178	32
	Proteus mirabilis CECT 170	32
	Pseudomonas aeruginosa ATCC 9027	64
	Salmonella typhimurium ATCC16028	32
	Serratia marcenses CECT 274	32
	Mycobacterium phlei ATCC 41423	2
Fungi	*Aspergillus niger* ATCC14604	32
	Aureobasidium pullulans ATCC 9348	16
	Gliocadium virens ATCC 4645	32
	Chaetonium globosum ATCC 6205	16
	Penicillium chrysogenum CECT 2802	128
	Penicillium funiculosum CECT 2914	16
Yeast	*Candida albicans* ATCC 10231	16
	Rhodotorula rubra CECT 1158	16
	Saccharomyces cerevisiae ATCC 9763	32

The use of the invention relates to cationic preservatives derived from the condensation of fatty acids and esterified dibasic amino acids, according to the following formula:



where:

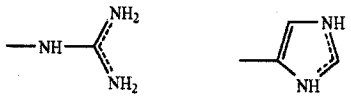
X^- is Br^-, Cl^-, or HSO_4^-

R1: is a linear alkyl chain from a saturated fatty acid or hydroxyacid from 8 to 14 atoms of carbon bonded to the α-amino acid group through an amidic bond.

R_2: is a linear or branched alkyl chain from 1 to 18 carbon atoms or an aromatic group.

R_3: is:

$-NH_3$



and n can be from 0 to 4.

The most preferred compound of the above class of compounds is LAE.

It is preferred to dissolve the compound directly before use in one of the following preferred solvents of food grade: water, ethanol, propylene glycol, isopropyl alcohol, other glycols, mixtures of glycols and mixtures of glycols and water. If the treatment shall be performed at a specific pH value the use of a corresponding buffer solution may be recommendable.

The composition optionally comprises auxiliary components and excipients. Such auxiliary components and excipients can be thickening agents (e.g. xanthan gum, guar gum, modified starches), anti-foam agents (e.g. dimethylpolysiloxane, silicon dioxide), products to obtain the optimal pH value (e.g. phosphates, tartrates, citrates, lactates), colouring agents (e.g. curcumin, tartrazine, erythrosine), and aroma products. It is preferred, that the preservative composition comprises LAE in an amount of from 0,0001% to 1% by weight relative to the whole weight of the preservative composition.

It is particularly preferred to use the inventive composition for the preservation of meat products, like for instance meat, poultry products, fish, crustaceans, vegetables, greens, emulsions, sauces, confectionery, bakery, pre-cooked meals, ready-to-serve meals, dairy products, egg-based products, jams, jellies, beverages, juices, wines and beers.

Moreover, the intended use relates to: wine-based flavoured drinks including products; non-alcoholic flavoured drinks; liquid tea concentrates and liquid fruit and herbal infusion concentrates; Barley Water; fruit and citric juices; Capilé Groselha; grape juices, unfermented, for sacramental use; wines, alcohol-free wines, fruit wines (including alcohol-free), alcoholic drinks with fruit; made wines, fruit sparkling wines, ciders, beers and perries (including alcohol-free); fermentation vinager; sod, saft; mead; spirits with less than 15% alcohol by volume; fillings of ravioli and similar products; quince, jams, jellies, marmelades and other fruit based spreads, candied, crystallized and glacé fruit and vegetables; sugar, glucose syrup, molasses and other sugars; transformed and dried fruits and vegetables, Frugtgrod and Rote Grütze, fruit and vegetable preparations (including fruit-based sauces); vegetable flesh; shell fruits; mousse, compote; salads, fruits and similar products, canned or bottled; Mostarda Di Fruta; Mascarpone; fruit based cake fillings; fruit gelling extracts and liquid pectine; vegetables and fruits in vinegar, brine or oil; rehydrated dried fruits; dressed dried fruits; sweetcorn canned in vacuum; potato dough and prefried, sliced, transformed, frozen, deep-frozen and peeled potatoes; dehydrated potato flakes and granulated (?); gnocchi; polenta; olives and olive-based preparations; jelly coating of meat products (cooked, cured or dried); burger meat; heat-treated meat products, sausages, breakfast sausages, pickled porks, pates, Foie Gras, Foie Gras Entier, Blocs de Foie Gras; Sagu; Mehu and Makeutettu; Ostkaka; Pasha; Semmelknodelteig; Polsebrod and bollery Dansk; canned

Flutes; gelatine; collagen based covers with a water activity of more than 0.6; salted meats, cured placenta, dried meat products; semi-preserved fish products including fish roe products, pickling, salted, dried fish, shrimps, cooked, Crangon crangon and Crangon vulgaris cooked; fresh, cooked, frozen and deep-frozen crusteacean; cheese, pre-packed, sliced, unripened and cured cheese, processed cheese, layered cheese and cheese with added foodstuffs; superficial treatment of cheese, fruits and vegetables; cheese substitute, meat substitute, fish substitute, crusteacean substitute; non-heat-treated dairy-based desserts, curdled milk, semolina and tapioca based desserts; liquid egg (white, yolk or whole egg), dehydrated, concentrated, frozen and deep-frozen egg products; pre-packed and sliced bread and rye-bread; partially baked, pre-packed bakery wares intended for retail sale, fine bakery wares with a water activity of more than 0.65; low-energetic bread; dry-biscuits; cereal- or potato-based snacks and coated nuts; batters, confectionery, glucose syrup based confectionery, flour based confectionery with a water activity of more than 0.65, chewing gum; Christmas pudding, nougats and marzipans; clotted cream; toppings (syrups for pancakes, flavoured syrups for milkshakes and ice cream, similar products), fat emulsions, dressing salads, emulsified sauces, non-emulsified sauces; prepared salads, mustard, seasonings and condiments; liquid soups and broths; aspic, liquid dietary food supplements; pearl barley; dietetic foods intended for special medical purposes and starches; dietetic formulae for weight control intended to replace total daily food intake or an individual meal; and other food products where the use of preservatives became necessary and allowed by law.

The cationic preservative may be added to a final stage of the product to be preserved or it may be added to a initial stage which would have the advantage of treating the food product, whereby it may be added as dry product to the product to be preserved, or in the form of a solution or dispersion.

The food products according to the invention are prepared according to the techniques which are well known to a person skilled in the art.

Procedures to Determine the Microbiological Population and Preservative Effect

The determination of the microbiological population is carried out according to the ISO standards.

The samples are treated by dilution in buffer peptone with the appropriate neutralising agent of the preservative. The culture media used for counting the microorganisms are: Soya triptone agar (32-35° C., 48 hours) for the determination of mesophilic bacteria; Sabouraud agar with chloramfenicol (25° C., 3-5 days) for fungi and yeast; Violet red bile glucose agar (32-35° C., 24 hours) for enterobacteria; Soya triptone agar (17° C., 5 days) for psychrotrophic bacteria.

EXAMPLES

Different examples of food products and formulations are shown where the product has been assayed. These examples are a part of the preparations and formulations assayed.

Example 1

This example shows the use of LAE in semi-preserved codfish in oil (table 2). The sample LAE was added to the oil assayed at a concentration of 100 ppm and its microbiological evolution at 4° C. was compared against a control.

TABLE 2

		Time (days)					
		0		14		43	
		Microorganism					
		Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast
Sample	Control (cfu/g)	$3.4 \cdot 10^3$	$4.0 \cdot 10^2$	$3.8 \cdot 10^5$	$2.0 \cdot 10^4$	$2.7 \cdot 10^8$	$1.2 \cdot 10^7$
	With LAE (cfu/g)	$7.6 \cdot 10^3$	$3.0 \cdot 10^2$	$1.0 \cdot 10^4$	$5.4 \cdot 10^3$	$8.5 \cdot 10^4$	$8.2 \cdot 10^3$

Example 2

This example shows the use of IAE in a chicken product (table 3). The sample LAE was added to at a concentration of 150 ppm and the evolution of aerobe and psychrotrophic bacteria at 10° C. was compared against a control.

TABLE 3

		Time (days)					
		0		14		43	
		Microorganism					
		Aerobe Bacteria	Psychro. Bacteria	Aerobe Bacteria	Psychro. Bacteria	Aerobe Bacteria	Psychro. Bacteria
Sample	Control (cfu/g)	$3.1 \cdot 10^5$	$2.4 \cdot 10^4$	$9.8 \cdot 10^5$	$6.5 \cdot 10^5$	$7.5 \cdot 10^8$	$4.2 \cdot 10^8$
	With LAE (cfu/g)	$1.2 \cdot 10^5$	$3.0 \cdot 10^4$	$4.2 \cdot 10^5$	$7.1 \cdot 10^4$	$6.1 \cdot 10^5$	$6.8 \cdot 10^4$

Example 3

This example shows the use of LAE in a carbonated orange beverage (table 4). The sample LAE was added to at a concentration of 100 ppm and its micobiological evolution at 17° C. was compared against a control.

TABLE 4

		Time (days)			
		0		14	
		Microorganism			
		Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast
Sample	Control (cfu/g)	$4.0 \cdot 10^2$	<10	$6.5 \cdot 10^4$	$1.7 \cdot 10^3$
	With LAE (cfu/g)	$4.3 \cdot 10^2$	<10	$1.0 \cdot 10^2$	<10

Example 4

This example shows the use of LAE in a blackberry juice (table 5). The sample LAE was added to at a concentration of 60 ppm and its micobiological evolution at 34° C. was compared against a control.

TABLE 5

		Time (days)			
		0		14	
		Microorganism			
		Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast
Sample	Control (cfu/g)	$5.1 \cdot 10^2$	<10	$2.5 \cdot 10^5$	$3.7 \cdot 10^4$
	With LAE (cfu/g)	$4.0 \cdot 10^2$	<10	$2.4 \cdot 10^3$	<10

Example 5

This example shows the use of LAE in custard (table 6). The sample LAE was added to at a concentration of 100 ppm and its micobiological evolution at 25° C. was compared against a control.

TABLE 6

		Time (days)			
		0		5	
		Microorganism			
		Aerobe Bacteria	Anaerobe Bacteria	Aerobe Bacteria	Anaerobe Bacteria
Sample	Control (cfu/g)	<10	<10	$9.1 \cdot 10^7$	$3.4 \cdot 10^7$
	With LAE (cfu/g)	<10	<10	$1.1 \cdot 10^3$	$4.1 \cdot 10^2$

Example 6

This example shows the use of LAE in fairy cakes (table 7). The sample LAE was added to at a concentration of 80 ppm and its micobiological evolution at 25° C. was compared against a control.

TABLE 7

		Time (months)			
		0		3	
		Microorganism			
		Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast
Sample	Control (cfu/g)	<10	<10	$9.1 \cdot 10^4$	$3.4 \cdot 10^3$
	With LAE (cfu/g)	<10	<10	$1.1 \cdot 10^2$	<10

Example 7

This example shows the use of LAE in veal stew (table 8). The sample LAE was added to at a concentration of 100 ppm and its microbiological evolution at 10° C. was compared against a control.

TABLE 8

		Time (days)					
		0			14		
		Microorganism					
		Aerobe Bacteria	Mould & yeast	Entero-bacteria	Aerobe Bacteria	Mould & yeast	Entero-bacteria
Sample	Control (cfu/g)	<10	<10	<10	$9.1 \cdot 10^4$	$3.4 \cdot 10^3$	$1.1 \cdot 10^2$
	With LAE (cfu/g)	<10	<10	<10	<10	<10	<10

Example 8

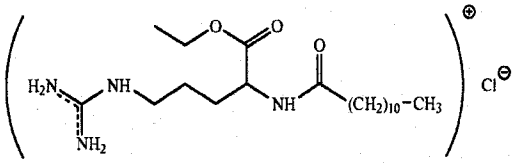
This example shows the use of LAE in ketchup (table 9). The sample LAE was added to at a concentration of 100 ppm and its microbiological evolution at 25° C. was compared against a control.

TABLE 9

		Time (days)					
		0			14		
		Microorganism					
		Aerobe Bacteria	Mould & yeast	Entero-bacteria	Aerobe Bacteria	Mould & yeast	Entero-bacteria
Sample	Control (cfu/g)	<10	<10	<10	$1.2 \cdot 10^6$	$4.3 \cdot 10^2$	$1.4 \cdot 10^3$
	With LAE (cfu/g)	<10	<10	<10	$2.2 \cdot 10^3$	$1.4 \cdot 10^1$	<10

The invention claimed is:

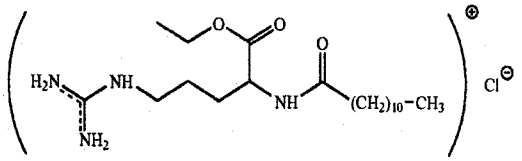
1. A food product containing a cationic preservative which is the ethyl ester of lauramide of arginine hydrochloride, derived from the condensation of fatty acids and esterified dibasic amino acids, said ethyl ester of lauramide of arginine hydrochloride having the following formula:



wherein said cationic preservative is present in the food product in an amount from about 0.006% to about 0.015% by weight.

2. The food product according to claim 1, wherein said cationic preservative is present in the food product in an amount from 0.008% to 0.015% by weight.

3. A method of preservation of a food product comprising the step of adding a cationic preservative which is the ethyl ester of lauramide of arginine hydrochloride, derived from the condensation of fatty acids and esterified dibasic amino acids, said ethyl ester of lauramide of arginine hydrochloride having the following formula:



wherein said cationic preservative is added to the food product as a solution, dispersion or solid before, during and/or after the manufacture of the food product at a concentration of from about 0.006% to about 0.015% by weight.

4. The method of claim **3** wherein the food product comprises fish, crustaceans, fish substitutes or crustacean substitutes.

5. The method of claim **3** wherein the food product comprises meat, meat substitutes or poultry products.

6. The method of claim **3** wherein the food product comprises vegetables, greens, sauces or emulsions.

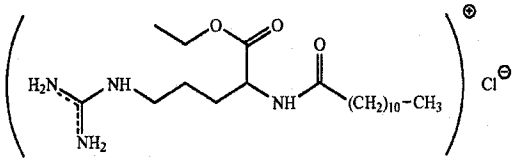
7. The method of claim **3** wherein the food product comprises beverages, juices, wines or beers.

8. The method of claim **3** wherein the food product comprises dairy, egg-based, jam, jelly, bakery or confectionary products.

9. The method of claim **3** wherein the food product comprises pre-cooked meal or ready-to-serve meal products.

10. The method according to claim **3**, wherein said ethyl ester of lauramide of arginine hydrochloride is added to the food product to provide a concentration of from 0.008% to 0.015% by weight.

11. A method of preservation of food products, wherein a cationic preservative which is the ethyl ester of lauramide of arginine hydrochloride, derived from the condensation of fatty acids and esterified dibasic amino acids, said ethyl ester of lauramide of arginine hydrochloride having the following formula:



wherein said cationic preservative is applied by surface treatment before, during and/or after the manufacture of the food product at a concentration of from about 0.006% to about 0.015% by weight.

12. A method according to claim **11**, wherein the cationic preservative is applied by spraying.

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